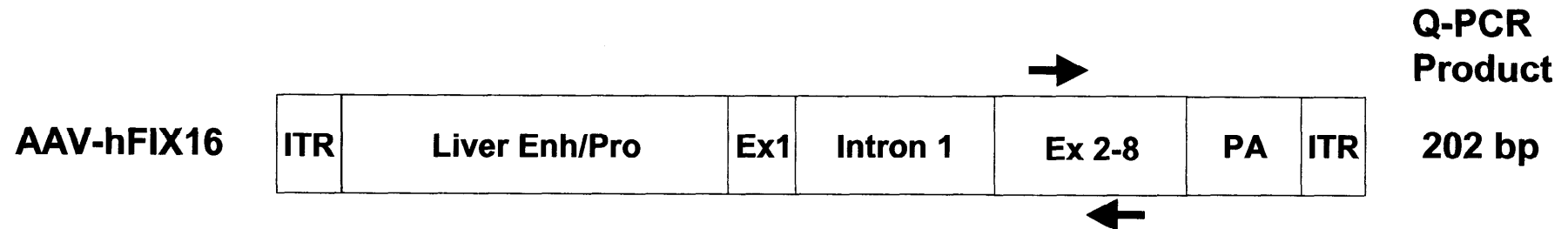


# Safety Studies to Support Intrahepatic Delivery of AAV

<b>Species</b>	<b>Analysis</b>
<b>Mice</b>	<b>Biodistribution</b> (10 tissues)
<b>Rat</b>	<b>Toxicology</b> (hematology, serum chemistry, histopathology) <b>Biodistribution</b> (8 tissues)
<b>Dog</b>	<b>Toxicology</b> (hematology, serum chemistry, histopathology) <b>Biodistribution</b> (liver, spleen, gonad, semen)
<b>Rabbit</b>	<b>Biodistribution</b> (gonad, total semen, fractionated semen, blood)
<b>Monkey</b>	<b>Toxicology</b> (hematology, serum chemistry, histopathology)

# Biodistribution Study in Rats

(Vector/# cells or ml blood)



Study Design			Day 50			Day 92		
Group	Treatment	Dose	Blood	Gonads	Liver	Blood	Gonads	Liver
1	Excipient		--	--	--	--	--	--
2	AAV-Null	1x10 <sup>13</sup> /kg	--	--	--	--	--	--
3	AAV-hFIX 15	1x10 <sup>11</sup> /kg	--	--	1/59	--	--	1/909
4	AAV-hFIX 15	1x10 <sup>12</sup> /kg	2.1x10 <sup>3</sup>	1/1x10 <sup>4</sup>	1/9	--	--	1/67
5	AAV-hFIX 15	1x10 <sup>13</sup> /kg	3.7x10 <sup>3</sup>	1/1.7x10 <sup>3</sup>	1/1.6	--	1/4.3x10 <sup>3</sup>	1/3.7

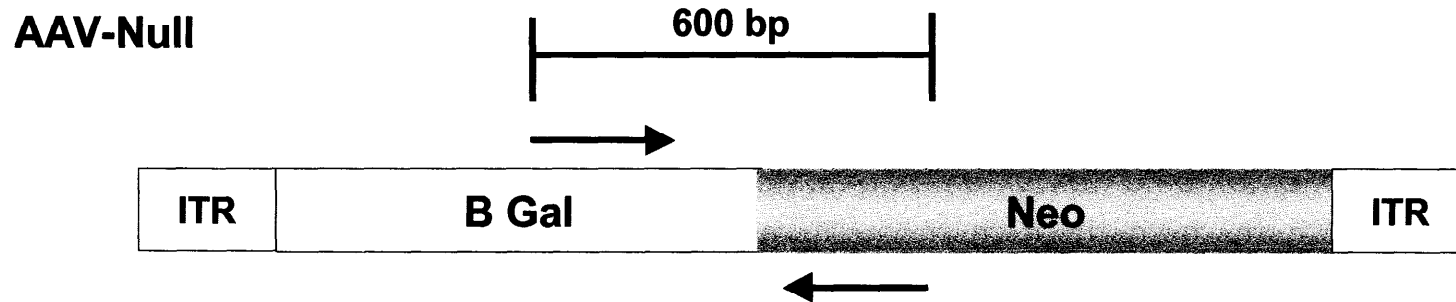
-- below sensitivity of assay (1/1.5x10<sup>4</sup> cells)

# Gonadal Distribution Study in Dogs

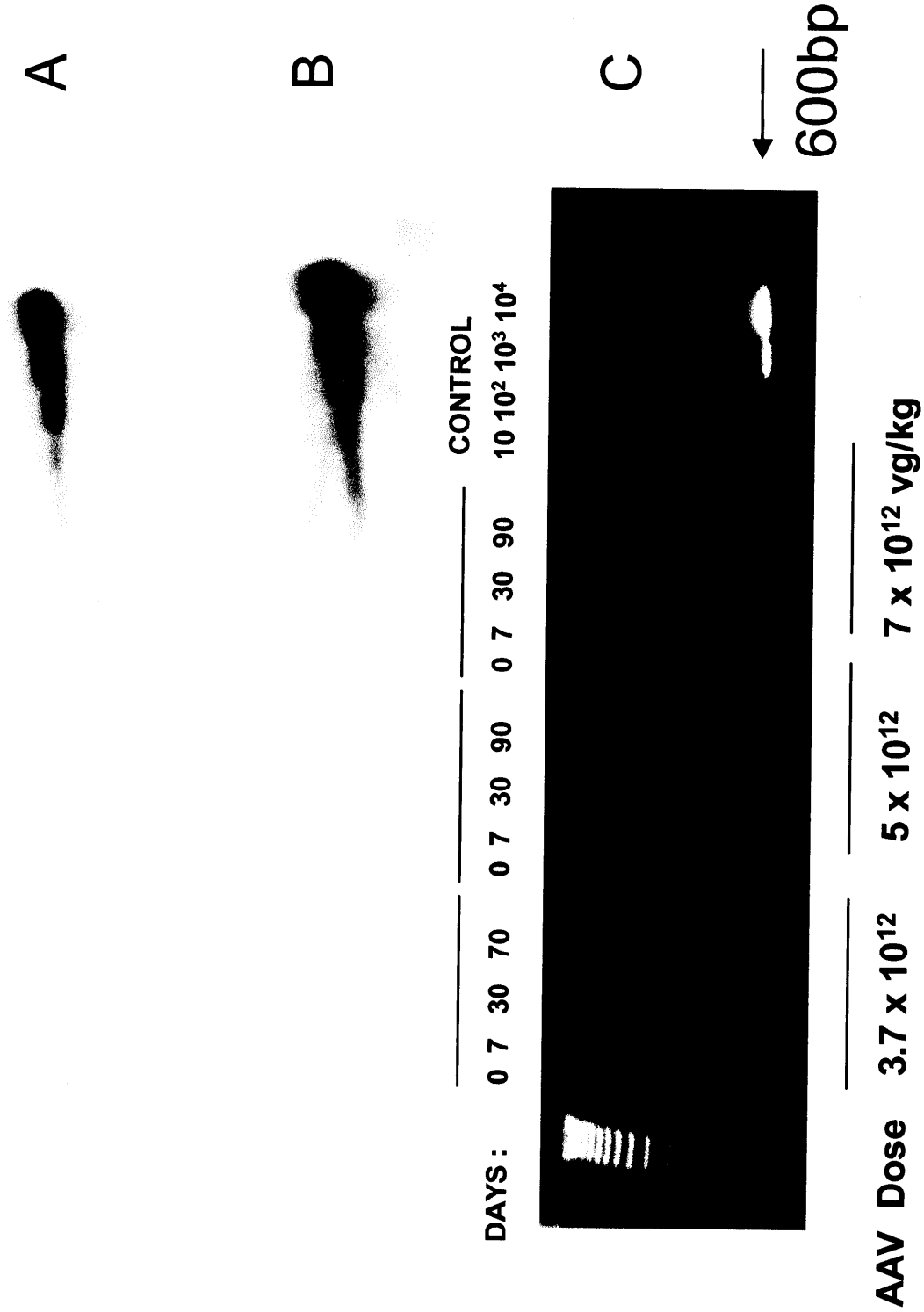
<u>No. Dogs</u>	<u>Vector</u>	<u>Dose/kg</u>	<u>Route</u>	<u>Semen (days)</u>
3	AAV-Null	3.7-7x10 <sup>12</sup>	HA-catheter via fluoroscopic guidance	0,7,30,70-90

## Analysis:

Toxicology: serum chemistry, hematology, histopathology  
PCR on gonadal tissue and semen



# Southern Blot Analysis of PCR Products from Dog Semen



# Toxicity/Biodistribution Study in Non-Human Primates

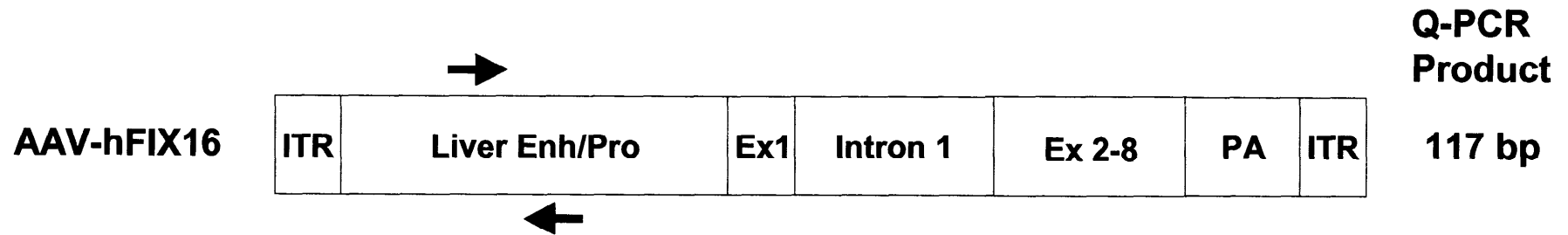
## Study Design

Group	Animals	Treatment	Dose/kg	Route	Sacrifice (days)
1	2	Excipient	--	HA	135
2	2	AAV-hFIX16	7x10 <sup>12</sup>	HA	135
3	2	AAV-hFIX16	7x10 <sup>12</sup>	PV	135

## Analysis:

Toxicology - hematology, serum chemistry, histopathology (liver)  
Biodistribution – liver and gonads  
IF of testes

# Biodistribution Study in Non-Human Primates (Vector / # cells)



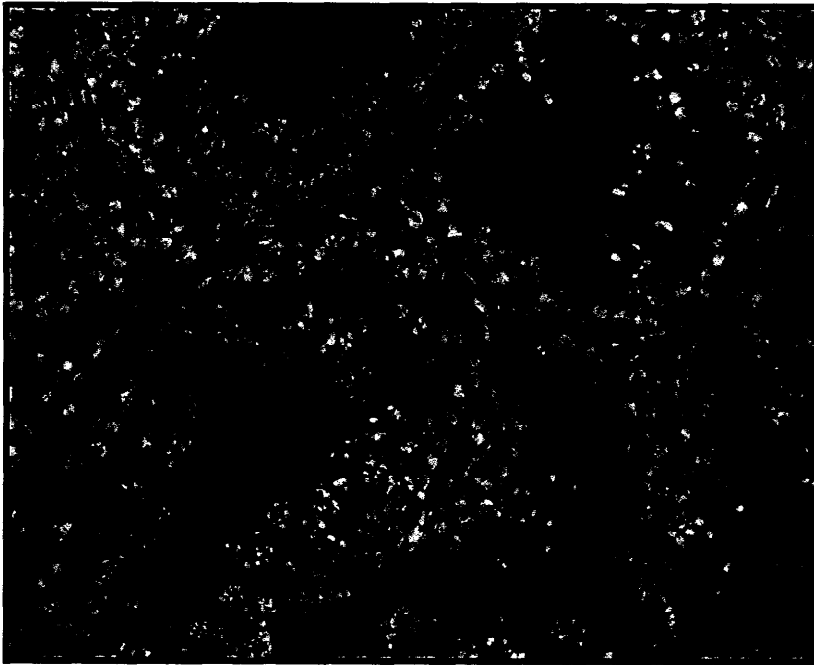
Study Design		
Animal	Treatment	Dose
1	Excipient/HA	
2	Excipient/HA	7x10 <sup>12</sup> /kg
3	Vector/HA	7x10 <sup>12</sup> /kg
4	Vector/HA	7x10 <sup>12</sup> /kg
5	Vector/PV	7x10 <sup>12</sup> /kg
6	Vector/PV	7x10 <sup>12</sup> /kg

Day 135	
Gonads	Liver
--	--
--	--
--	--
--	1 / 2.6
--	1.6 / 1
--	--

-- below sensitivity of assay (1/80,000 cells)

# HSPG Staining in Non-Human Primate Testis

HSPG IF (red) with DAPI counterstaining (blue)



200x



630x

- No HSPG (AAV receptor)- Immunoreactivity in the spermatogonia or Sertoli cells
- HSPG was observed in the connective tissue of the testis.

# **Experiments to Assess Horizontal and Vertical Germline Transmission**

## **Horizontal transmission:**

- Favre et al (Mol Ther 4:559-566; 2001)**
- Development of cell-based infectivity assay to assess biological activity of AAV in semen samples**

## **Vertical transmission:**

- Expose murine sperm cells to AAV-FIX, perform IVF and assess risk of AAV infection and integration into the male germ line**



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# **Biodistribution Study of Biologically Active rAAV in Non-Human Primates**

Favre et al Mol Ther 4:559-566; 2001

## **Study Design**

<b>Animals</b>	<b>Treatment</b>	<b>Dose (IU)/kg</b>	<b>Route</b>	<b>Timepoints</b>
8	AAV-Epo	$5 \times 10^8$ - $1 \times 10^{10}$	IM	30 min, 1-7 day, monthly

## **Analysis:**

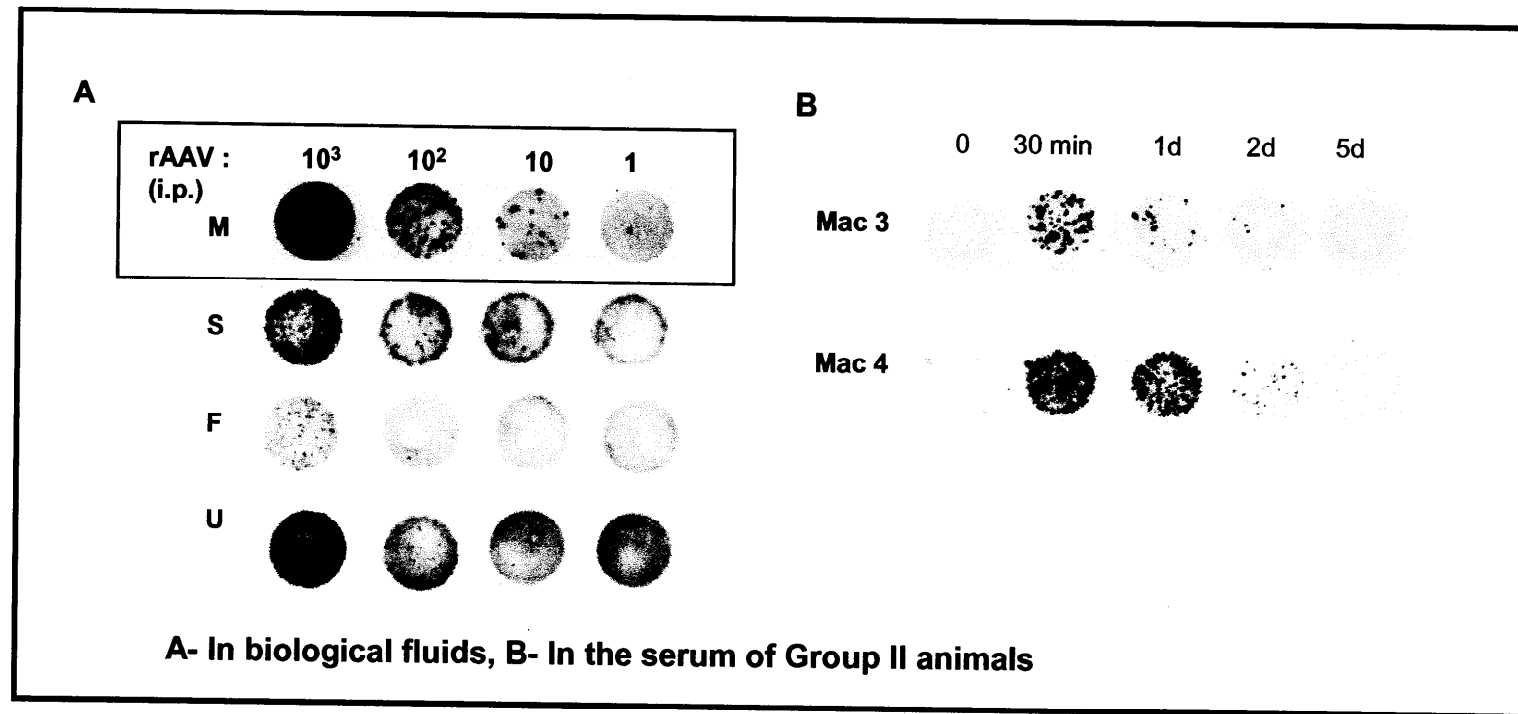
**Body fluids (serum, feces, urine, saliva, lacrymal, nasal, but not semen) - PCR and  
Replication Center Assay**

**PBMC - PCR**

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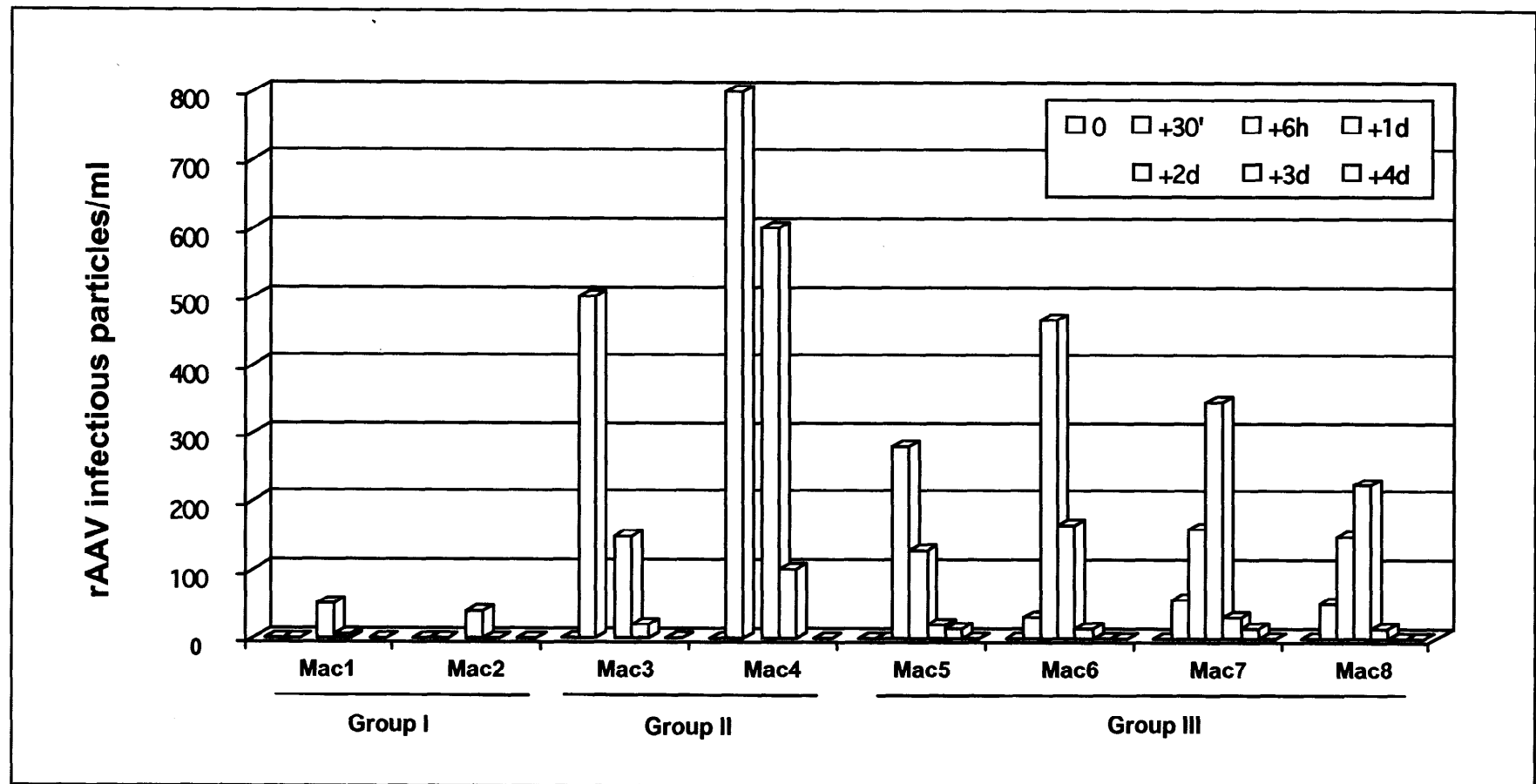
# Detection of Biologically Active rAAV in Sera

Favre et al Mol Ther 4:559-566; 2001



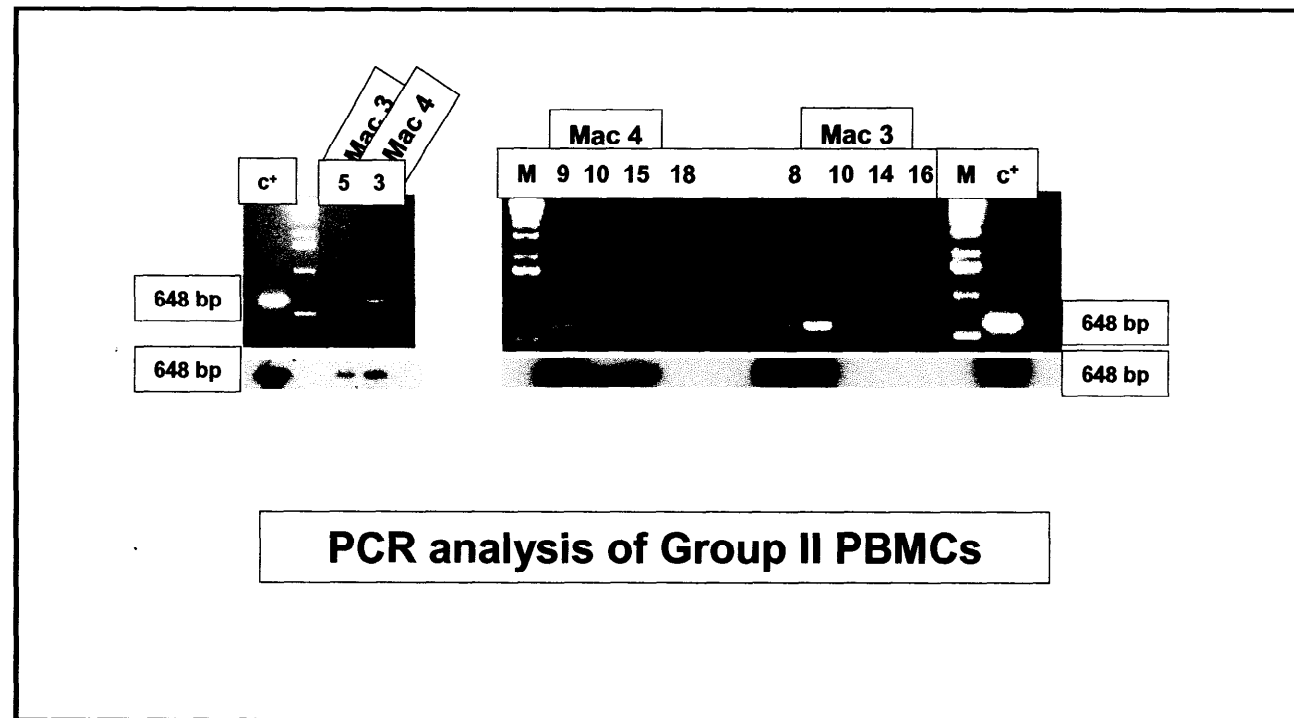
# Kinetics of Biologically Active rAAV in Sera

Favre et al Mol Ther 4:559-566; 2001



# Detection of rAAV Sequences in PBMCs

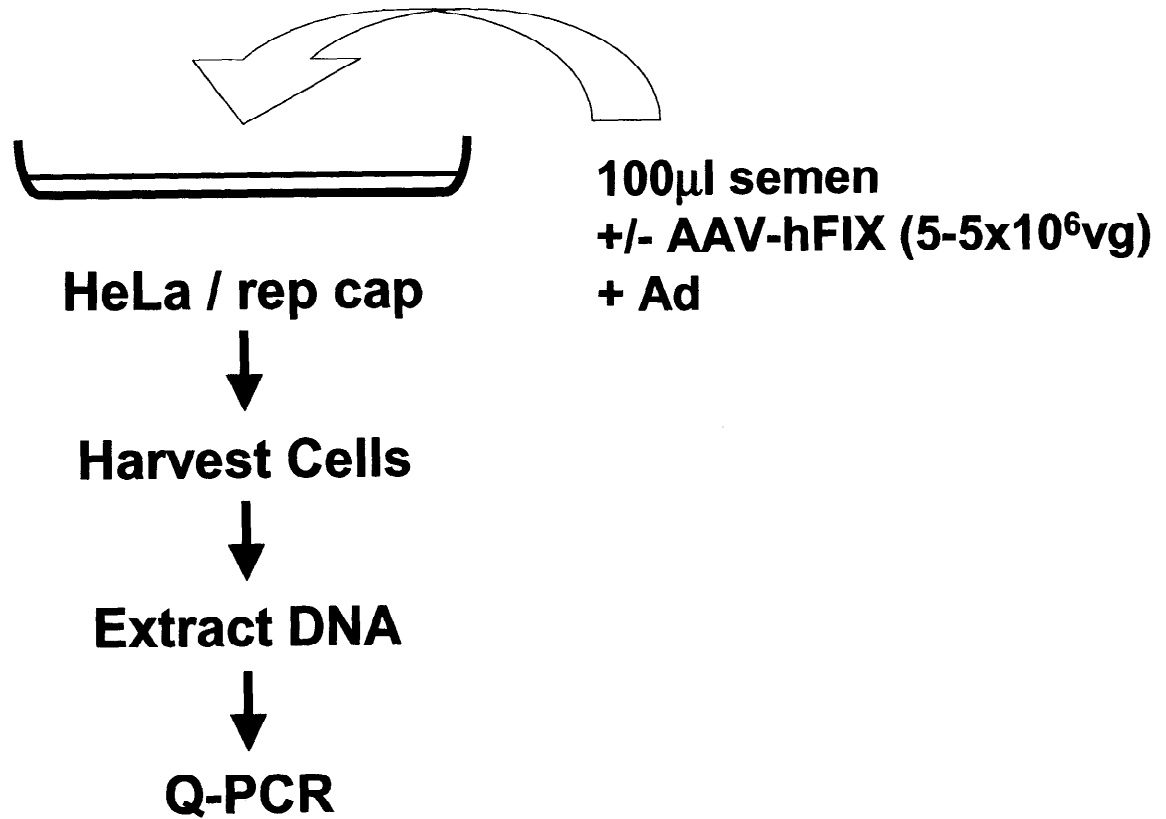
Favre et al Mol Ther 4:559-566; 2001































# Summary of Favre Data

- **rAAV vector sequences are detected in all body fluids for approximately 6 days**
- **PCR signal is due to packaged rAAV sequences rather than free DNA**
- **Biologically active rAAV vectors were detected only in serum for 48-72 hrs, suggesting that risk of horizontal transmission is limited to a short period of time post-injection**
- **Vector can be detected in PBMCs for up to 10 months following IM administration**

# Infectivity Assay Development



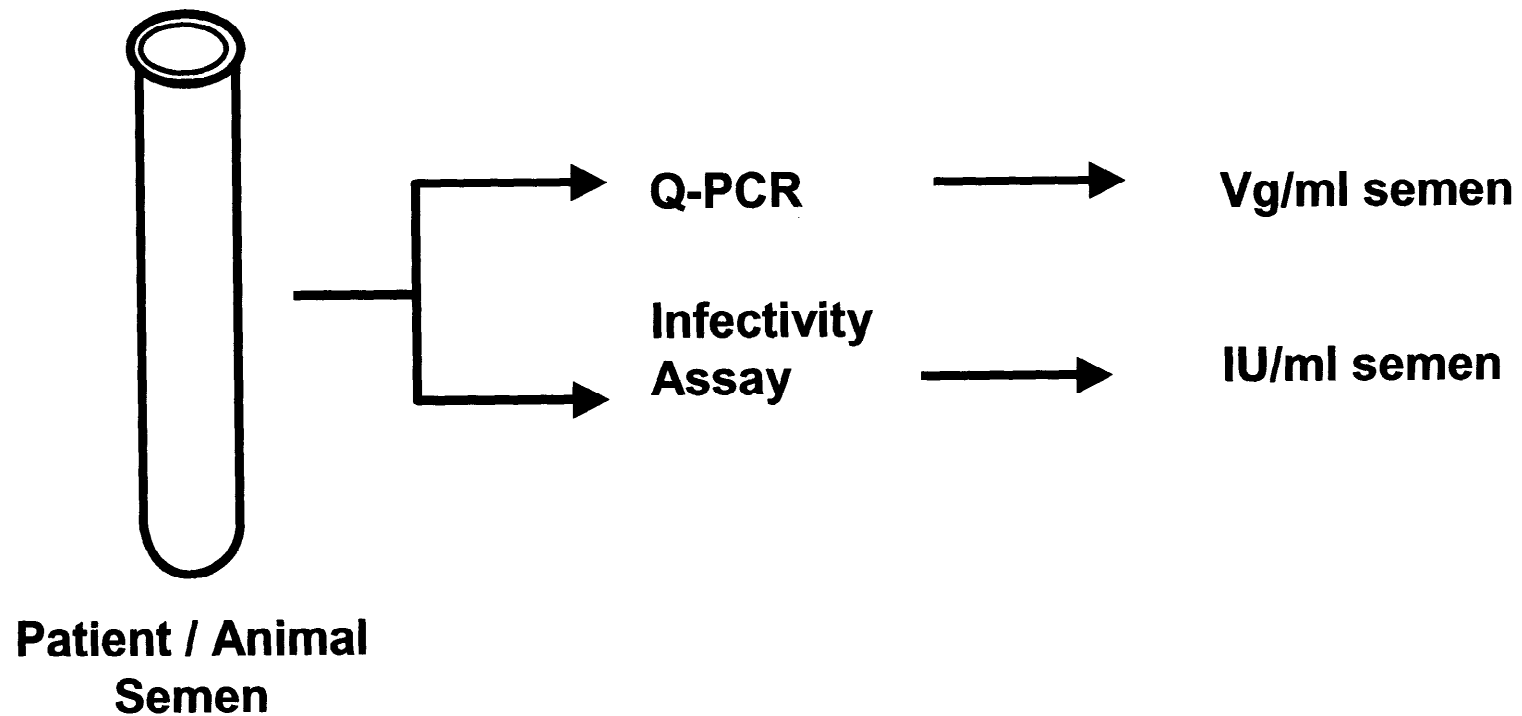
# Infectivity Assay Development

<u>Ad</u>	<u>Vector</u>	<u>In Semen</u>		<u>In Media</u>	
+	$5 \times 10^6$				
+	$5 \times 10^5$				
+	$5 \times 10^4$				
+	$5 \times 10^3$				
+	$5 \times 10^2$				
+	$5 \times 10^1$				
+	0				

Lowest dose that results in amplification signal in media – 10-50 vg/well

Lowest dose that results in amplification signal in semen – 500 vg/well/100 $\mu$ l  
5000 vg/ml semen

# Semen Analysis



**Compare kinetics of clearance of physical and infectious particles**



# **Vertical Transmission: AAV Transduction of Sperm/IVF**

- 1) Isolate murine sperm**
- 2) Expose to AAV-hFIX**
- 3) In vitro fertilization**
- 4) Implant fertilized oocytes into pseudopregnant females**
- 5) Isolate fetuses at 10-12 day gestation**
- 6) Extract DNA/ Southern blot analysis**
- 7) Single Copy of AAV-hFIX used as evidence of vertical germline transmission**

# Summary

- **Extent of vector dissemination to animal tissues correlates with dose and decreases with time**
- **Following intrahepatic delivery of rAAV, vector is either absent from gonadal tissue (dogs, non-human primates) or present at levels 1000 x lower than liver (rats) and clears with time**
- **Studies in NHP suggest AAV in serum is not infectious after 72 hr, but vector signal can be detected in PBMCs for up to 10 months after IM administration**
- **AAV receptor (HSPG) is not expressed on non-human primate spermatogonial cells**
- **Data consistent with hematogenous dissemination of vector to gonads with clearance over time**

# **Germline Transmission Issues being Addressed**

**Is there infectious virus in semen?**

**Infectivity Assay**

**Human/Animals**

**Are vector sequences in semen  
associated with motile sperm, other  
cells or seminal fluid?**

**Fractionation**

**Human/Animals**

**Can AAV infect mature and/or  
immature spermatogonial cells?**

**IVF Expts**

**Animals**